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BLOOD LYMPHOCYTE SUBPOPULATIONS AND ANTIBODY-DEPENDENT, CELL-MEDIATED CYTOTOXICITY (ADCC) IN ALOPECIA AREATA AND UNIVERSALIS

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Abstract. T-lymphocyte subpopulations and antibody-dependent, cell-mediated cytotoxicity (ADCC) were studied in patients with alopecia areata and universalis. The proportions of T-cells with the receptors for IgG (Tg) and T-cells with receptor for IgM (Tm) were determined in 16 cases. The ADCC of unfractionated lymphocytes was examined in 5 cases. A higher proportion of Tg-cells (T-cells with low affinity for sheep red cells) and a more pronounced ADCC of the peripheral lymphocytes were found in patients with alopecia areata and universalis than in age- and sex-matched normal donors.

Key words: T-lymphocyte; Suppressor cells; Killer cells

Treatment with corticosteroids, topically (18) or systematically (4), with topical application of contact allergens (8) and with PUVA (22) can induce regrowth of hair in patients with alopecia areata (AA) and totalis (AT). The recent therapeutical findings with skin sensitizers and with PUVA have awakened new interest in the immunologic background of this disease. Immunological studies have shown that the proportion of circulating T-cells, rosetting with sheep red blood cells (SRBC) not treated with neuraminidase, is significantly lower in patients with AA than in normal persons (2, 6) and that the number of T-cells in peripheral blood increased significantly after topical application of DNCB (16). Histopathologically, AA is characterized by mainly lymphocytic inflammatory infiltrates around bulbs and follicles, especially in the early stages of the disease (20). These cells display epidermotropism, suggesting that they are T-cells (7). It has been proposed that this may reflect an immune response to some hypothetical hair-associated antigen (9).

In view of the above-mentioned findings it was found of interest to study some further aspects of immunological reactivity in AA. Recently, subpopulations of human T-lymphocytes having receptors for the Fc portion of either IgG (Tg) or IgM (Tm) have been demonstrated by rosetting techniques (5, 12, 13). Tm-cells have been shown to exert helper functions and Tg-cells are also active in antibody-dependent cell-mediated cytotoxicity (ADCC).

In the present study we have determined the proportions of Tg and Tm-cells and the ADCC of unfractionated lymphocytes from patients with AA and alopecia universalis (AU) as well as in age- and sex-matched healthy controls.

MATERIALS AND METHODS

Patients

Sixteen patients with alopecia areata (AA) and universalis (AU) were studied. Brief data concerning sex, age, different types of alopecia, history of atopy, values of IgE, findings of auto-antibodies and treatment given are shown in Table I.

Immunological methods

Cell separations. Heparinized venous blood was centrifuged on a Ficoll-Isopaque gradient (3). Macrophages were removed with a magnet after treatment of the cell suspension with carbonyl iron or by adherence to plastic Petri dishes (5). T-enriched fractions were separated by rosetting with neuraminidase-treated sheep red blood cells (SRBC) followed by gradient centrifugation (11). The SRBC attached to T-cells were lysed by treatment with 0.83% NH_4Cl. The final pellet contained 95–100% of E-rosette forming cells.

Determination of Tg and Tm-cells

Tg and Tm-cells were identified by rosette formation as described by Moretta and co-workers (5, 12, 13). Details are given elsewhere (1). Briefly, Tg-cells were identified by their ability to form rosettes with bovine erythrocytes coated with rabbit antibovine erythrocyte IgG, and Tm-cells by their reaction with bovine erythrocytes coated with rabbit anti-bovine erythrocyte IgM. A rosette was

Table I. *Patients with Alopecia Areata*

AA=alopecia areata: patchy loss of scalp hair, AT=alopecia totalis: total loss of scalp hair, AU=alopecia universalis: total loss of body hair, DLE=discoid lupus erythematosus

Case no.	Sex	Born Age (y.)	Case History	History of atopy[a]	IgE (mg/l)[b]	Auto-antibodies	Therapy
1 AR	F	1931 49	1965 AA. 1966 AT. 1967 AU.	–	50	ANA: 1/10	–
2 BC	F	1928 52	1968 AA. 1969 AT. 1970 AU.	+	10	0	–
3 BR	F	1945 35	1975 AA. Same year AU. DLE on face since 1963.	–	30	0	–
4 LPO	M	1961 19	1971–75 AA. 1975 AU.	+	476	0	–
5 LB	M	1941 39	1955 AA. 1955: Regrowth. 1976 AA. Same year AU.	+	35	Sm. muscle 1/10 Glomerul. 1/10	–
6 BK	M	1948 32	1961-68 AA. 1968 Regrowth. 1971–78 AA: 1978 AU.	+	26	0	PUVA Sept. 79– April 80
7 JAK	M	1949 31	1964 diffuse hair loss. 1964 Regrowth. 1977 diffuse hair loss. 1978 AU.	+	145	0	PUVA Nov. 79- June 80
8 TL	M	1946 34	1978 AA. 1979 AU.	–	21	0	–
9 ACS	F	1953 27	1979 AA. 1980 AU.	+	7	Gastric parietal cells: 1/100	PUVA
10 AP	F	1959 21	1979 AA. 1980 AU.	–	116	0	–
11 CA	M	1945 35	1978 AA. 1979 AT. 1980 AU.	–	12	0	–
12 AL	F	1961 19	1974 AA.	+	40	0	–
13 ML	F	1942 38	1953 AU. 1956 Regrowth. 1977 AA.	+	220	0	PUVA Nov. 79– April 80
14 BP	F	1934 46	1971 AA. 1975 Regrowth. 1979 AA.	+	96	Thyreoidea 1/10	–
15 LL	F	1926 54	1973 AA. 1973 Regrowth. 1979 AA.	–	22	0	–
16 YA	F	1944 36	1979 AA.	–	41	0	–

[a] History of asthma, hayfever and/or atopic dermatitis.
[b] IgE, normal limits: 0-400 mg/litre.

defined as a lymphocyte with three or more red cells attached. Four hundred lymphocytes were counted for rosette formation. All tests were performed in duplicate.

Assay of antibody-dependent cell-mediated cytotoxicity (ADCC)

The technique was described in detail by Wasserman et al. (21). Briefly, $Na_2{}^{51}CrO_4$-labelled chicken erythrocytes (Ch-RBC) served as target cells, rabbit anti-ChRBC serum as anti-target serum. 0.5×10^6 purified non-fractionated lymphocytes were mixed with 5×10^4 isotope-labelled Ch-RBC in RPMI with 5% FCS (Fetal Calf Serum). The lymphocyte-erythrocyte ratio thus obtained was 25: 1. The final dilution of rabbit anti-ChRBC serum which gave optimal cytotoxicity was 10^{-6}. 0.5 ml of this anti-serum dilution was added to the cell suspensions. Normal rabbit serum was used as control. Labelled erythrocytes and rabbit anti-ChRBC serum constituted another control. All tests were performed in duplicate. The lymphocyte-anti-ChRBC serum mixtures were incubated at 37°C in a 5% CO_2 atmosphere for 20 h. Cytotoxicity was expressed as the percentage of total radioactivity released into the supernatant.

Statistical analysis

The statistical method used was Student's *t*-test.

Table II. *Percentages of total T-cells (Neuraminidase-treated SRBC rosette-forming cells)* TG and TM cells in Alopecia Areata patients

Patients				Controls		
Case no.	Total T-cells	TG	TM	Total T-cells	TG	TM
1	74	27	62	78	17	52
2	80	39	52	76	16	52
3	76	18	58	80	17	49
4	80	20	40	80	24	48
		(31)[a]	(49)		(18)	(43)
5	80	39	47	84	25	59
6	69	31	70	64	16	52
7	77	18	59	87	22	51
8	87	31	52	90	20	43
9	76	50	44	78	23	43
10	83	36	63	87	23	57
11	80	20	37	81	17	43
		(30)[a]	(42)		(21)	(36)
12	73	20	54	72	21	61
13	76	26	50	82	20	39
14	76	34	54	80	27	51
15		41	47		22	33
16	76	29	34	78	22	42
Mean±S.D.	78±4.3	30±9.5	51±9.8	80±6.3	21±3.4	48±7.5
P value	>0.05	<0.01	>0.05			

[a] Experiments made on different occasions.

RESULTS

Lymphocyte subpopulations

The total number of peripheral blood lymphocytes of the patients was as that of healthy persons (data not presented).

The overall proportion of T-cells (rosetting with neuraminidase-treated SRBC, including both high and low affinity T-lymphocytes) was the same for both patients and normal subjects. The results of the determination of Tg and Tm-cells are given in Table II. Carbonyl iron and adherence methods were both used for removing phagocytes. It was found that there was no difference between the two methods (to be published). In 16 samples the patients with AA and AU showed significantly higher proportions of Tg-cells than normal donors. On the other hand, there was no significant difference in the proportions of Tm-cells between the patients and normal controls.

Table III. *ADCC of lymphocytes and proportion of Tg cells in AA patients and normal controls*

	Patients		Controls	
Case no.	% Tg	% specific isotope release[a]	% Tg	% specific isotope release
2	39	55	16	39
4	31	59	18	47
5	39	62	25	51
6	31	50	16	39
11	30	59	18	47
Mean±S.D.		55±6.2		43±5.7

P-value (difference patients/controls) <0.05.
[a] Lymphocyte–erythrocyte ratio 25 : 1.

Antibody-dependent cell-mediated cytotoxicity

The results of experiments on ADCC of unfractionated lymphocytes from 5 patients and normal controls are shown in Table III. Target cell damage indicated by ^{51}Cr release was expressed as a percentage of specific isotope release from the target cell, i.e. isotope release in the presence of anti-target cell serum minus isotope release in the presence of normal rabbit serum (21).

ADCC of lymphocytes from AA patients was significantly higher than the corresponding value for the normal donors (Table IV).

DISCUSSION

The role of lymphocytes in connection with the pathogenesis of AA has been discussed in recent

years. It has been demonstrated that the proportion of T-cells rosetting with sheep red blood cells (SRBC) not treated with neuraminidase, i.e. T-cells with high affinity for SRBC, is reduced in patients with this disease. This finding may be of interest in view of the fact that AA is frequently observed in diseases associated with immunodeficiency (9) and that subtle immune defects have been reported in patients with AA (10). However, there are no published reports about different T-cell subsets in AA. According to our findings, patients with AA and AU have significantly higher proportions of Tg cells than healthy controls. As the lymphocyte counts in AA and AU were within the normal range, the absolute numbers of Tg cells in these patients must also be high as compared with age-matched controls. The frequency of Tg-cells was the same irrespective of the method used for depletion of macrophage–monocytes. This is of interest in view of the fact that cells of monocyte lineage have Fc receptors for IgG and often contaminate lymphocyte suspensions. As the yields obtained by phagocytosis and adherence technique, respectively, do not completely overlap, the use of both these methods greatly adds to the accuracy of monocyte elimination when identifying cells with Fc receptor.

Present data do no contradict the findings regarding the low proportion of T lymphocytes with high affinity receptors for SRBC found in AA (2, 6). On the contrary, the increased frequency of Tg cells, which are known to have low affinity receptors for SRBC (i.e. rosetting with neuraminidase-treated SRBC) and the normal overall proportion of T-cells (both low and high affinity cells) suggest that in our material too the proportion of T lymphocytes with high affinity receptors was reduced.

The surface markers used in the present investigation distinguish fairly well between functionally different lymphocyte fractions. Thus, Tg cells are known to mediate ADCC, whereas Tm cells are considered to lack this activity. Our results demonstrate that ADCC of unfractionated lymphocytes was significantly higher in AA and AU patients than in controls, which suggests that there is concordance between the results on surface markers and on effector functions. However, as the lymphocyte population active in ADCC includes both T- and non-T-cells, it cannot be excluded that the proportion of non-T-cells mediating ADCC is increased in AA and AU or alternatively, that these cells are more active.

It has also been shown that Tg lymphocytes exert a suppressor activity modulating the PWM-induced Ig synthesis in vitro (14). Results from our experiments (to be published) suggest that this suppressor activity might be increased in AA and AU. If verified, the finding corresponds well to the results on receptor studies presented in this paper.

This is of particular interest, as it has been demonstrated recently by means of monoclonal antibodies secreted by hybridoma that the majority of Tg cells reacted with antibodies that defined antigen presented on monocytes and granulocytes, implying that these cells were instead of monocyte–myeloid lineage (19). However, according to L. Moretta (Communication IV Int. Congress of Immunology, Paris, 1980) 35–70% of lymphocytes reacting with neuraminidase-treated SRBC and having Fc receptors for IgG are true T-cells. The above cited results on increased suppression of Ig production in vitro suggest that lymphocytes which are more frequent in AA and AU are suppressor cells of Tg type.

The implications of the present findings are unknown. If AA really is an autoimmune disease, one would rather expect a decreased suppressor activity, as has been described in states of autoimmunity. Our data tend to argue against the concept of autoimmunity and tally better with the idea of AA as being associated with immunodeficiency, since in some types of this disease increased proportions of Tg cells and suppressor activity have been reported (15). On the other hand, Tg cells are known to mediate ADCC and natural killer cell (NK) activity (17) and it is possible that in AA these cells are involved in some kind of cytotoxic reaction—possibly of an autoimmune character—against hair roots or follicles. The increased cytotoxic potential demonstrated in this study could reflect this type of reactivity.

In some patients, Tg cells were no more numerous than in controls (patients 3, 7, 12) or, alternatively different results were obtained at different times of examination (patients 4, 11). Patient no. 3 had had discoid lupus erythematosus (DLE) for 17 years and in our experience patients with both SLE and DLE have low T suppressor cells counts. Patient no. 7 had been successfully treated with PUVA for more than 6 months prior to examination. Reports from lymphocyte studies on PUVA-treated patients have shown a decrease in thymus-derived E-rosette-forming cells. So far, no studies

on T cell subpopulations during PUVA-treatment seem to have been made.

Patients 12 and 14 both had a mild form of AA. whereas patients 15 and 16 had the same type of AA and elevated Tg cells. Patients 4 and 11 had different Tg cell counts on two different occasions and it is possible that the proportion of Tg cells could change during the course of AA, being related to the pathodynamics of the disease. However, this has not yet been studied.

A history of atopy is recorded for all these patients. There was no correlation between positive or negative history of atopy and the level of Tg. The same is valid also for the values of IgE and the findings of autoantibodies.

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